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# Post-antibiotic effect of marbofloxacin, enrofloxacin and amoxicillin against selected respiratory pathogens of pigs

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**Abstract**: The post-antibiotic effect is defined as the period of bacterial growth suppression that persists after a limited exposure of organisms to antimicrobials and knowledge of its duration is important in establishing and optimising current dosing schedules for the treatment of bacterial infections. The post-antibiotic effect of marbofloxacin, enrofloxacin and amoxicillin were evaluated *in vitro* for *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis* and *Pasteurella multocida* strains which originated from clinical samples of diseased pigs and were confirmed as susceptible to all tested antimicrobials based on determination of minimal inhibitory concentrations. The post-antibiotic effect for individual antimicrobials was monitored at five and ten times minimum inhibitory concentrations for one and two hours. The duration of the post-antibiotic effect for tested antimicrobials was found to exhibit the following order for all tested pathogens: marbofloxacin > enrofloxacin > amoxicillin. The longest duration of post-antibiotic effect of all tested antimicrobials was found in *A. pleuropneumoniae* and the shortest post-antibiotic effect duration was detected in *P. multocida*. No statistical differences in post-antibiotic effect duration were found within marbofloxacin and enrofloxacin in *A. pleuropneumoniae* and *H. parasuis* strains. In *P. multocida* strains there was a statistically significant difference ($P = 0.0189$). On the other hand, the differences between amoxicillin and marbofloxacin or enrofloxacin were statistically significant in all cases ($P$-values ranged between 0.0058 and 0.008). The prolonged post-antibiotic effect of fluoroquinolones and amoxicillin on important Gram-negative swine pathogens was confirmed. The results can be used to clarify the effect and mechanism of action of antimicrobial drugs in veterinary medicine.

**Keywords**: antimicrobials; antimicrobial treatment; antimicrobial susceptibility testing; infection of swine; PAE duration

*Actinobacillus pleuropneumoniae*, *Pasteurella multocida* and *Haemophilus parasuis* are well-known pathogens from the family *Pasteurellaceae* that may cause severe illness and increased mortality of pigs of all ages. They can affect the respiratory tract of animals and *H. parasuis* may be involved in the development of a serious systemic disease called Glässer's disease (Biberstein 1990; Nedbalcova et al. 2006). These pathogens have the ability to cause diseases with a negative economic impact, resulting in large losses for pig breeders. Losses are often due to high morbidity and mortal-

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ity in acute forms of illness or decreased growth rate during fattening that characterise chronic infections, as well as the high costs of antimicrobial treatment for sick pigs. Based on the results of *in vitro* susceptibility testing of *A. pleuropneumoniae*, *P. multocida* and *H. parasuis* to antimicrobials, the aminopenicillins (amoxicillin), and fluoroquinolones appear to be suitable for the treatment of infections caused by these pathogens in the field due to the high efficiency and relatively low level of resistance. However, some of the commonly used antimicrobials for treatment of respiratory infections of pigs are becoming less effective due to increased resistance among bacterial pathogens in recent years (Aarestrup et al. 2004; Cai et al. 2005; De la Fuente et al. 2007; Nedbalcova and Kucerova 2013; de Jong et al. 2014; Nedbalcova et al. 2014; El Garch et al. 2016; El Garch et al. 2017; Nedbalcova et al. 2017; Sweeney et al. 2017). The number of effective treatment options is limited, partially due to the recent regulations, such as in the case of third- and fourth-generation cephalosporines, which are coming under more scrutiny due to the associated evolution of extended-spectrum beta-lactamase resistance genes. Therefore, these agents have been voluntarily banned in some European Union member states, including Denmark, The Netherlands and France.

The post-antibiotic effect (PAE) is defined as the period of bacterial growth suppression that persists after a limited exposure of organisms to antimicrobials (Craig and Gudmundsson 1996). The PAE may last after drug levels are no longer detectable, and thus knowledge of the duration of PAE may be of important clinical interest in establishing and optimising current dosing schedules for the treatment of infections (Craig 1993; Craig and Gudmundsson 1996; Carbone et al. 2001). There is a general lack of this information for antimicrobials and important pathogens in veterinary medicine. The study of PAE provides information about the action of antibiotics that cannot be derived from standard *in vivo* sensitivity tests. The effects found in *in vitro* testing may be nullified by a variety of *in vivo* factors that are intrinsic to the drug, the host and the strain. In general, a long *in vitro* PAE is highly probable to be predictive of a favourable outcome, whereas use of drugs with shorter PAE can be predictive of a poor clinical response. The PAE is also considered to be an important parameter besides standard minimum inhibitory concentrations, completing the pharmacokinetic/pharmacodynamic profile of antimicrobials (Craig 1993; Carbone et al. 2001). Earlier studies of the PAE performed on Gram-negative bacteria exposed to β-lactam antibiotics have generally not demonstrated any PAE effect with the exception of imipenem and carbapenem. A rather minor effect of β-lactam antibiotics on PAE duration in Gram-positive bacteria (staphylococci, streptococci) was demonstrated with an extension of PAE duration by addition of clavulanic acid (Gould et al. 1991; Craig and Gudmundsson 1996). On the other hand, fluoroquinolones elicit a prolonged growth suppression in Gram-negative bacteria such as *E. coli* (Rescott et al. 1988). To our knowledge, there is no available data related to the determination of the PAE of the above-mentioned antimicrobials against the respiratory pathogens of pigs.

The aim of this study was to evaluate the *in vitro* post-antibiotic effect (PAE) of amoxicillin, enrofloxacin and marbofloxacin against selected *A. pleuropneumoniae*, *P. multocida* and *H. parasuis* isolates originating from diseased pigs from Czech farms.

## MATERIAL AND METHODS

**Isolates**. Thirty isolates of each pathogen (*A. pleuropneumoniae*, *H. parasuis* and *P. multocida*; in total 90 isolates) were obtained *post mortem* from the lungs of diseased pigs from farms in the Czech Republic. The isolates were provided by an animal diagnostic laboratory (Sevaron, Czech Republic) and they were identified using MALDI-TOF.

**Antimicrobials**. The following antimicrobials were used in the study: marbofloxacin, enrofloxacin and amoxicillin (Discovery Fine Chemicals, United Kingdom).

**Minimum inhibitory concentrations determination**. The minimum inhibitory concentrations (MIC) of marbofloxacin, enrofloxacin and amoxicillin were determined for thirty isolates of each pathogen using a microdilution method according to documents of the Clinical and Laboratory Standards Institute (CLSI 2013a; CLSI 2013b). Briefly, the MICs were determined using microtitre panels made in the microbiology laboratory of the authors (Veterinary Research Institute, Brno, Czech Republic) with Mueller Hinton Broth (BD Company, USA) for the testing of *P. multocida* iso-

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lates or veterinary fastidious medium (prepared in-house at the Veterinary Research Institute according to CLSI instructions) for *A. pleuropneumoniae* and *H. parasuis* isolates. The plates were inoculated with two-fold serial dilution ranges of the tested antimicrobials (marbofloxacin, enrofloxacin, amoxicillin). The concentrations of antimicrobials were between 0.002–64 mg/l. The quality of MIC determination was controlled with control *E. coli* (ATCC 25922) and *A. pleuropneumoniae* (ATCC 27090) reference strains. Susceptibility to the tested antimicrobials was determined based on clinical breakpoints (CLSI 2013b). The breakpoints valid for swine respiratory pathogens are defined only for enrofloxacin (susceptible ≤ 0.25 mg/l; intermediate 0.5 mg/l; resistant ≥ 1 mg/l). The breakpoints of marbofloxacin were derived from breakpoints determined for dogs and cats (susceptible ≤ 1 mg/l; intermediate 2 mg/l; resistant ≥ 4 mg/l), and breakpoints for amoxicillin were derived from breakpoints of ampicillin (susceptible ≤ 0.5 mg/l; intermediate 1 mg/l; resistant ≥ 2 mg/l). Ten isolates selected for PAE determination were susceptible to the tested antimicrobials with the lowest possible MIC.

**PAE determination**. The determination of PAE was accomplished using methods described by Craig and Gudmundsson (1996). The tested *P. multocida* strains were inoculated into tubes with 20 ml Mueller Hinton broth (MHB; BD Company, USA), *A. pleuropneumoniae* strains were inoculated with MHB supplemented with 10% NAD (MHB NAD; Sigma-Aldrich, USA) and *H. parasuis* strains were inoculated with MHB NAD with 5% of lysed horse blood (MHB NAD LHB; LabMediaServis, Czech Republic). The tubes were incubated overnight at 37°C. The optical density of each culture tube at 580 nm was adjusted to 0.3. One millilitre of the adjusted culture was individually added to 9 ml of previously prepared medium containing antimicrobials – marbofloxacin, enrofloxacin and amoxicillin – and to 9 ml of drug-free control medium. Final bacterial concentrations ranged from $10^6$ to $10^7$ CFU/ml. Bacterial cultures were exposed to the antimicrobials in concentrations of 5 × and 10 × MIC for one and two hours.

After the exposure period, the cultures were diluted 1 : $10^3$ into MHB (*P. multocida*) or MHB NAD (*A. pleuropneumoniae*) or MHB NAD LHB (*H. parasuis*). The PAE was quantified by counts of viable colonies before and after antibiotic exposure and then hourly for 24 h. Viable colonies were counted after overnight incubation of samples taken every hour on blood agar plates (*P. multocida*) or chocolate agar plates (*A. pleuropneumoniae* and *H. parasuis*) (LabMediaServis, Czech Republic). The PAE was expressed in hours and determined by the formula:

$$PAE = T - C \quad (1)$$

where:

PAE – post-antibiotic effect;

*T* – the time required for the count of CFU in the test culture to increase by 1 $\log_{10}$ (10-fold) above the count observed immediately after antibiotic removal;

*C* – the time required for the count of CFU in an untreated culture to increase by 1 $\log_{10}$ above the

Table 1. The minimum inhibitory concentration (MIC; mg/l) values of selected isolates to the tested antimicrobials

| Strain No. | *A. pleuropneumoniae* MIC | | | *P. multocida* MIC | | | *H. parasuis* MIC | | |
|---|---|---|---|---|---|---|---|---|---|
| | MAR | ENR | AMX | MAR | ENR | AMX | MAR | ENR | AMX |
| 1 | 0.03 | 0.03 | 0.125 | 0.015 | 0.008 | 0.125 | 0.06 | 0.015 | 0.06 |
| 2 | 0.03 | 0.03 | 0.5 | 0.015 | 0.008 | 0.125 | 0.03 | 0.015 | 0.06 |
| 3 | 0.03 | 0.03 | 0.5 | 0.015 | 0.008 | 0.125 | 0.03 | 0.015 | 0.06 |
| 4 | 0.03 | 0.03 | 0.5 | 0.015 | 0.008 | 0.125 | 0.015 | 0.015 | 0.06 |
| 5 | 0.03 | 0.03 | 0.5 | 0.015 | 0.008 | 0.125 | 0.03 | 0.015 | 0.06 |
| 6 | 0.03 | 0.03 | 0.25 | 0.015 | 0.008 | 0.125 | 0.015 | 0.015 | 0.06 |
| 7 | 0.03 | 0.03 | 0.5 | 0.015 | 0.008 | 0.125 | 0.015 | 0.015 | 0.06 |
| 8 | 0.03 | 0.03 | 0.5 | 0.015 | 0.008 | 0.125 | 0.015 | 0.06 | 0.06 |
| 9 | 0.03 | 0.03 | 0.5 | 0.015 | 0.008 | 0.125 | 0.015 | 0.015 | 0.06 |
| 10 | 0.03 | 0.03 | 0.25 | 0.015 | 0.008 | 0.125 | 0.03 | 0.125 | 1 |

AMX = amoxicillin; ENR = enrofloxacin; MAR = marbofloxacin

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Table 2. Duration of the post-antibiotic effect (PAE; hours) in *A. pleuropneumoniae* strains ($n$ = 10)

| Exposure time | Marbofloxacin | | Enrofloxacin | | Amoxicillin | |
|---|---|---|---|---|---|---|
| | average PAE | range of PAE | average PAE | range of PAE | average PAE | range of PAE |
| 1 h (5 × MIC) | 8.3 | 8–10 | 8.3 | 6–9 | 4.0 | 2–6 |
| 1 h (10 × MIC) | 9.0 | 8–12 | 8.6 | 8–10 | 5.7 | 3–6 |
| 2 h (5 × MIC) | 10.1 | 8–12 | 9.9 | 8–10 | 6.6 | 4–10 |
| 2 h (10 × MIC) | 10.3 | 10–12 | 10.2 | 9–12 | 8.5 | 6–10 |

MIC = minimum inhibitory concentration

count observed immediately after completion of the same procedure used on the test culture without antimicrobials.

**Statistical analysis**. The Wilcoxon test was used to compare significant differences in the duration of PAE, with a $P$-value of 0.05 considered significant.

## RESULTS

The MIC values for marbofloxacin, enrofloxacin and amoxicillin against the tested *A. pleuropneumoniae*, *P. multocida* and *H. parasuis* strains are shown in Table 1. Only susceptible strains according to CLSI classification were selected, except strain No. 10, which was intermediate to amoxicillin.

The PAEs were calculated using the formula (1) PAE = $T - C$ (see Material and Methods). The $C$ value was one for cultures of all tested pathogens because the time required for the count of CFU in an untreated culture to increase by 1 $\log_{10}$ above the count observed immediately after completion of the same procedure used on the test culture without antimicrobials was exactly one hour. The duration of the PAE in bacterial cultures of *A. pleuropneumoniae* strains treated by different concentrations of antimicrobials for either one or two hours is shown in Table 2.

The duration of PAE mediated by the tested antimicrobials against all monitored pathogens was generally found to be longest for *A. pleuropneumoniae* strains. In the majority of *A. pleuropneumoniae* isolates, the average duration of PAEs was gradually prolonged depending on the length of exposure and the concentration of the tested antimicrobials. The average PAE of amoxicillin was shorter than the PAEs of the tested fluoroquinolones. Marbofloxacin elicited the most pronounced PAE in comparison to the other tested antimicro-

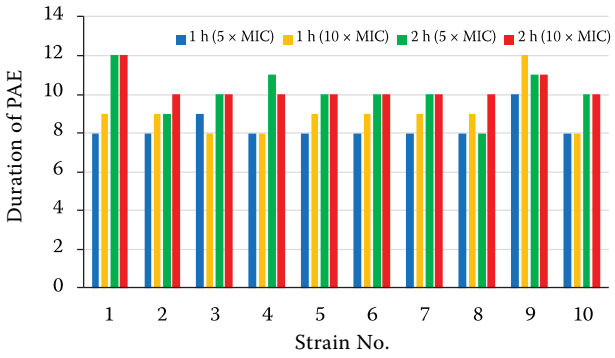


Figure 1. Post-antibiotic effect (PAE; hours) in *A. pleuropneumoniae* strains – marbofloxacin

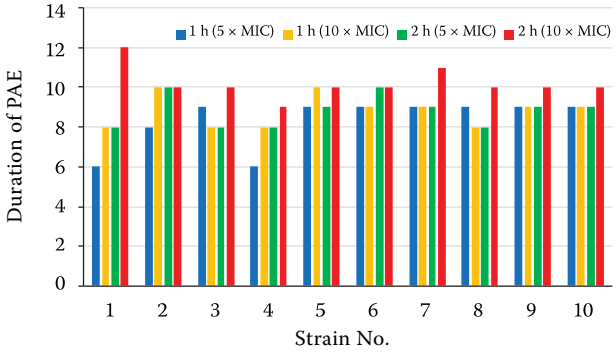


Figure 2. Post-antibiotic effect (PAE; hours) in *A. pleuropneumoniae* strains – enrofloxacin

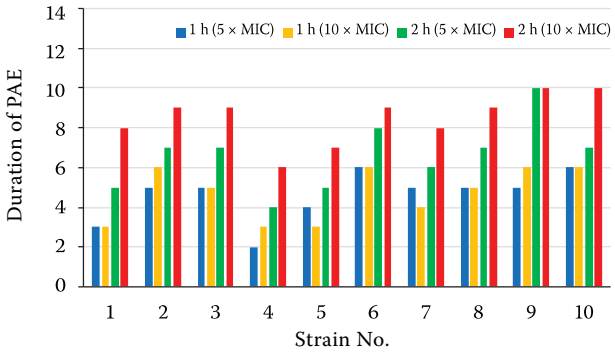


Figure 3. Post-antibiotic effect (PAE; hours) in *A. pleuropneumoniae* strains – amoxicillin

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Table 3. Statistical analysis of post-antibiotic effect duration in *A. pleuropneumoniae* strains

| Compared values | *P*-value | Statistical significance |
|---|---|---|
| MAR 5 × MIC, 1 h/ MAR 5 × MIC, 2 h | 0.0418 | * |
| MAR 5 × MIC, 1 h/ MAR 10 × MIC, 1 h | 0.0083 | ** |
| MAR 5 × MIC, 2 h/ MAR 10 × MIC, 2 h | 0.014 | * |
| MAR 10 × MIC, 1 h/ MAR 10 × MIC, 2 h | 0.5862 | ns |
| ENR 5 × MIC, 1 h/ ENR 5 × MIC, 2 h | 0.1983 | ns |
| ENR 5 × MIC, 1 h/ ENR 10 × MIC, 1 h | 0.1983 | ns |
| ENR 5 × MIC, 2 h/ ENR 10 × MIC, 2 h | 0.0126 | * |
| ENR 10 × MIC, 1 h/ ENR 10 × MIC, 2 h | 0.0126 | * |
| AMX 5 × MIC, 1 h/ AMX 5 × MIC, 2 h | 0.7656 | ns |
| AMX 5 × MIC, 1 h/ AMX 10 × MIC, 1 h | 0.0047 | ** |
| AMX 5 × MIC, 2 h/ AMX 10 × MIC, 2 h | 0.0047 | ** |
| AMX 10 × MIC, 1 h/ AMX 10 × MIC, 2 h | 0.0071 | ** |

AMX = amoxicillin; ENR = enrofloxacin; MAR = marbofloxacin; MIC = minimum inhibitory concentration; ns = not significant

*Significant; **highly significant

bials, although the difference compared to enrofloxacin was more modest. The duration of PAE for the individual tested *A. pleuropneumoniae* strains and individual pathogens is shown in Figures 1–3.

The statistical significance of the differences of PAE duration in all *A. pleuropneumoniae* strains depending on exposure time and the concentration of the tested antimicrobials is shown in Table 3.

The results in other tested pathogens are presented similarly to the *A. pleuropneumoniae* strains.

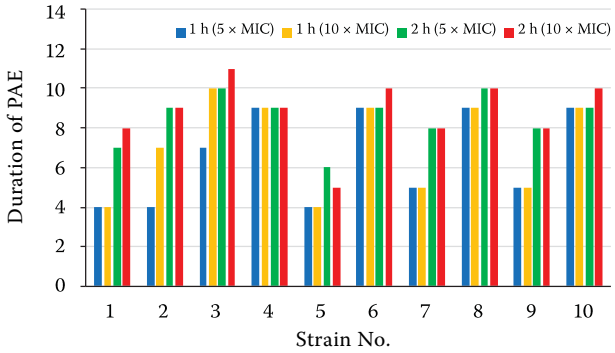


Figure 4. Post-antibiotic effect (PAE; hours) in *H. parasuis* strains – marbofloxacin

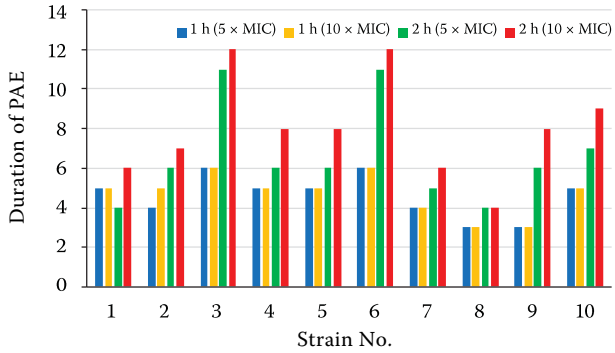


Figure 5. Post-antibiotic effect (PAE; hours) in *H. parasuis* strains – enrofloxacin

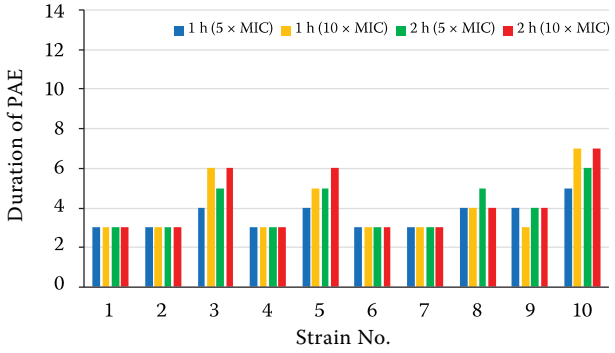


Figure 6. Post-antibiotic effect (PAE; hours) in *H. parasuis* strains – amoxicillin

Table 4. Duration of the post-antibiotic effect (PAE; hours) in *H. parasuis* strains ($n$ = 10)

| Exposure time | Marbofloxacin | | Enrofloxacin | | Amoxicillin | |
|---|---|---|---|---|---|---|
| | average PAE | range of PAE | average PAE | range of PAE | average PAE | range of PAE |
| 1 h (5 × MIC) | 6.5 | 4–9 | 4.6 | 3–6 | 3.6 | 3–5 |
| 1 h (10 × MIC) | 7.7 | 4–9 | 4.8 | 3–6 | 4.0 | 3–7 |
| 2 h (5 × MIC) | 8.7 | 6–10 | 6.6 | 4–11 | 4.0 | 3–6 |
| 2 h (10 × MIC) | 8.8 | 5–11 | 8.0 | 4–12 | 4.2 | 3–7 |

MIC = minimum inhibitory concentration

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The duration of the PAE in bacterial cultures of *H. parasuis* strains is shown in Table 4; the duration of PAE for individual tested *H. parasuis* strains and individual pathogens is shown in Figures 4–6, and the statistical significance of differences in PAE duration in all *H. parasuis* strains depending on exposure time and the concentration of tested antimicrobials is shown in Table 5.

Comparison of the results obtained for the three tested antimicrobials, revealed that PAE duration differed most in *H. parasuis* strains. The average PAE was found to be longest for marbofloxacin especially in response to 1-hour exposure. On the other hand, the highest PAE strain variability and highest absolute values were detected for 2-hour exposure to enrofloxacin. The dependence of PAE on exposure time and concentration was rather minimal in the case of amoxicillin.

The duration of the PAE in *P. multocida* strains treated with different concentrations of antimicrobials for one or two hours is shown in Table 6, and the duration of PAE for individual tested *H. parasuis* strains and individual pathogens is shown in Figures 7–9.

The shortest PAE for all tested antimicrobials was detected in *P. multocida*. Previous testing of antimicrobials identified marbofloxacin as having the best absolute PAE. Similar to *H. parasuis* strains, the highest variability in PAE duration depending on

Table 5. Statistical analysis of post-antibiotic effect duration in *H. parasuis* strains

| Compared values | *P*-value | Statistical significance |
|---|---|---|
| MAR 5 × MIC, 1 h/ MAR 5 × MIC, 2 h | 0.3458 | ns |
| MAR 5 × MIC, 1 h/ MAR 10 × MIC, 1 h | 0.0201 | * |
| MAR 5 × MIC, 2 h/ MAR 10 × MIC, 2 h | 0.0079 | ** |
| MAR 10 × MIC, 1 h/ MAR 10 × MIC, 2 h | 0.233 | ns |
| ENR 5 × MIC, 1 h/ ENR 5 × MIC, 2 h | 1 | ns |
| ENR 5 × MIC, 1 h/ ENR 10 × MIC, 1 h | 0.0131 | * |
| ENR 5 × MIC, 2 h/ ENR 10 × MIC, 2 h | 0.0058 | ** |
| ENR 10 × MIC, 1 h/ ENR 10 × MIC, 2 h | 0.0074 | ** |
| AMX 5 × MIC, 1 h/ AMX 5 × MIC, 2 h | 0.2652 | ns |
| AMX 5 × MIC, 1 h/ AMX 10 × MIC, 1 h | 0.0719 | ns |
| AMX 5 × MIC, 2 h/ AMX 10 × MIC, 2 h | 0.3458 | ns |
| AMX 10 × MIC, 1 h/ AMX 10 × MIC, 2 h | 0.4237 | ns |

AMX = amoxicillin; ENR = enrofloxacin; MAR = marbofloxacin; MIC = minimum inhibitory concentration; ns = not significant

*Significant; **highly significant

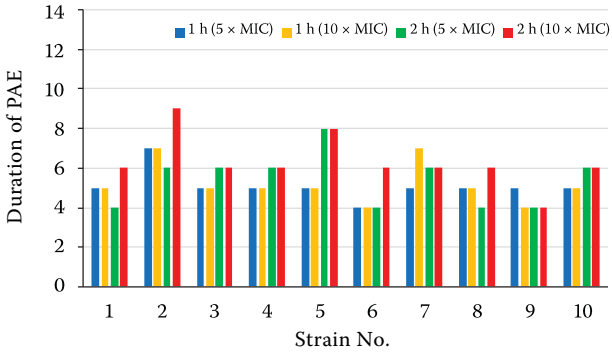


Figure 7. Post-antibiotic effect (PAE; hours) in *P. multocida* strains – marbofloxacin

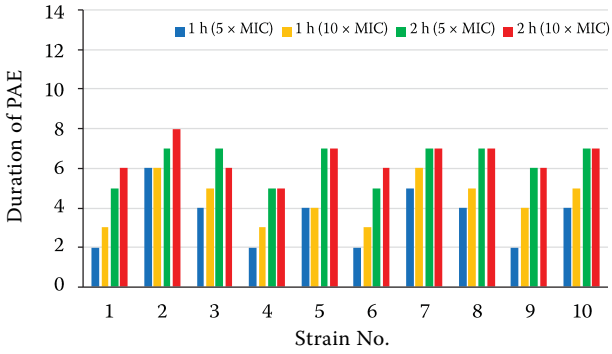


Figure 8. Post-antibiotic effect (PAE; hours) in *P. multocida* strains – enrofloxacin

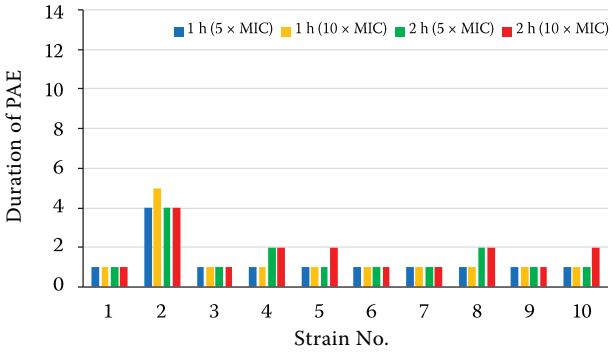


Figure 9. Post-antibiotic effect (PAE; hours) in *P. multocida* strains – amoxicillin

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Table 6. Duration of the post-antibiotic effect (PAE; hours) in *P. multocida* strains ($n$ = 10)

| Exposure time | Marbofloxacin | | Enrofloxacin | | Amoxicillin | |
|---|---|---|---|---|---|---|
| | average PAE | range of PAE | average PAE | range of PAE | average PAE | range of PAE |
| 1 h (5 × MIC) | 5.1 | 2–6 | 3.5 | 2–6 | 1.3 | 1–4 |
| 1 h (10 × MIC) | 5.6 | 3–6 | 4.4 | 3–6 | 1.3 | 1–4 |
| 2 h (5 × MIC) | 5.4 | 4–8 | 6.3 | 5–7 | 1.5 | 1–4 |
| 2 h (10 × MIC) | 6.3 | 4–9 | 6.5 | 5–8 | 1.7 | 1–4 |

MIC = minimum inhibitory concentration

exposure time and concentration of antimicrobials was found for enrofloxacin, with very low or almost no dependency in the cases of marbofloxacin and amoxicillin.

The statistical significance of differences of PAE duration in all *H. parasuis* strains depending on exposure time and concentration of tested antimicrobials is shown in Table 7.

Finally, we made a statistical comparison of average values of PAE duration within individual tested antimicrobials, separately for each pathogen. The statistical analysis is shown in Table 8.

No statistical differences in PAE duration were found within marbofloxacin and enrofloxacin in *A. pleuropneumoniae* and *H. parasuis* strains. In *P. multocida* strains there was a statistically significant difference ($P$ = 0.0189). On the other hand, the differences between amoxicillin and marbofloxacin or enrofloxacin were statistically significant in all cases ($P$-value was from 0.0058 to 0.008).

Table 7. Statistical analysis of post-antibiotic effect duration in *P. multocida* strains

| Compared values | $P$-value | Statistical significance |
|---|---|---|
| MAR 5 × MIC 1 h/ MAR 5 × MIC 2 h | 0.3458 | ns |
| MAR 5 × MIC 1 h/ MAR 10 × MIC 1 h | 0.0201 | * |
| MAR 5 × MIC 2 h/ MAR 10 × MIC 2 h | 0.0079 | ** |
| MAR 10 × MIC 1 h/ MAR 10 × MIC 2 h | 0.233 | ns |
| ENR 5 × MIC 1 h/ ENR 5 × MIC 2 h | 1 | ns |
| ENR 5 × MIC 1 h/ ENR 10 × MIC 1 h | 0.0131 | * |
| ENR 5 × MIC 2 h/ ENR 10 × MIC 2 h | 0.0058 | ** |
| ENR 10 × MIC 1 h/ ENR 10 × MIC 2 h | 0.0074 | ** |
| AMX 5 × MIC 1 h/ AMX 5 × MIC 2 h | 0.2652 | ns |
| AMX 5 × MIC 1 h/ AMX 10 × MIC 1 h | 0.0719 | ns |
| AMX 5 × MIC 2 h/ AMX 10 × MIC 2 h | 0.3458 | ns |
| AMX 10 × MIC 1 h/ AMX 10 × MIC 2 h | 0.4237 | ns |

AMX = amoxicillin; ENR = enrofloxacin; MAR = marbofloxacin; MIC = minimum inhibitory concentration; ns = not significant

*Significant; **highly significant

## DISCUSSION

The correct use of antimicrobial agents for the treatment of bacterial infections requires knowledge of the susceptibility of the specific strain to antimicrobial agents. When choosing the right therapeutic option, we should ideally know the MIC value as well as other parameters like pharmacokinetic/pharmacodynamic characteristics including possibly PAE, which provides information about

Table 8. Statistical evaluation of post-antibiotic effect duration within individual tested antimicrobials

| Pathogen | Compared values | $P$-value | Statistical significance |
|---|---|---|---|
| *Actinobacillus pleuropneumoniae* | MAR/ENR | 0.4364 | ns |
| | MAR/AMX | 0.0059 | ** |
| | ENR/AMX | 0.0058 | ** |
| *Haemophilus parasuis* | MAR/ENR | 0.2337 | ns |
| | MAR/AMX | 0.0059 | ** |
| | ENR/AMX | 0.0058 | ** |
| *Pasteurella multocida* | MAR/ENR | 0.0189 | * |
| | MAR/AMX | 0.008 | ** |
| | ENR/AMX | 0.0059 | ** |

AMX = amoxicillin; ENR = enrofloxacin; MAR = marbofloxacin; ns = not significant

*Significant; **highly significant

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the action of antibiotics, allowing us to determine the optimal dosing intervals. PAE is an important pharmacodynamic parameter for determining longer dosing intervals, reducing the adverse effect on organisms, reducing spread of antimicrobial resistance and reducing the cost of treatment (Craig and Gudmundsson 1996).

Aminopenicillins and fluoroquinolones are recommended antimicrobial agents used to control infections caused by respiratory and systemic pathogens of pigs such as *A. pleuropneumoniae*, *H. parasuis* and *P. multocida*; therefore, amoxicillin, enrofloxacin and marbofloxacin were chosen for evaluation of PAE as specific data for these antimicrobials are missing. Concentrations of 5 × MIC and 10 × MIC and exposure times of one and two hours were used. In our study marbofloxacin gave the longest PAEs for all three pathogens (5.1–10.3 h), followed by enrofloxacin (3.5–10.2 h). The shortest duration was recorded for amoxicillin (1.3–8.5 h), which was especially short for *P. multocida* (1.3–1.7 h).

Enrofloxacin was selected in our panel of tested antibiotics as representative of its class; it is registered for the treatment of respiratory pathogens of swine and is commonly used in the field. The clinical breakpoints of enrofloxacin for selected swine pathogens have been defined, and therefore it is used in pharmacokinetic/pharmacodynamic studies and MIC monitoring programs in swine medicine (Burch 2010; El Garch et al. 2016; Wang et al. 2016). It is known that part of enrofloxacin (51% in pigs) is metabolised into an active metabolite – ciprofloxacin, which needs to be taken into consideration in the case of estimation of the *in vivo* PAE effect along with other factors which might play role (Anadon et al. 1999; Trouchon and Lefebvre 2016). According to some studies, the duration of enrofloxacin PAE *in vivo* can be shorter than that of ciprofloxacin, based on a shorter elimination half-life ($T_{1/2}$) of the latter. However, most reports describe no significant differences in the $T_{1/2}$ values of ciprofloxacin and enrofloxacin (Nouws et al. 1988; Cester and Toutain 1997; Garcia Ovando et al. 1999; Rao et al. 2002; Idowu et al. 2010). The results of other studies indicated that ciprofloxacin levels (an active metabolite of enrofloxacin) were too low in the plasma of pigs and that enrofloxacin could serve as a marker for pharmacokinetic parameters calculation (Anadon et al. 1999; Messenger et al. 2012; Hao et al. 2013).

A significant concentration-dependent increase in the duration of PAE was detected for both fluoroquinolones. The same observation was demonstrated in other studies (Carbone et al. 2001; Zhao et al. 2014). In similar studies, the PAE effect of enrofloxacin was shorter compared with the results of our study – around three hours in Gram-negative bacteria (*E.coli*, *S. Typhimurium*, *P. multocida*) following a 2-hour exposure to 8 × MIC. Direct comparison with other studies is rather difficult due to differences in the characteristics of pathogens and strains (MIC and origin) and methodology (especially detection) (Wetzstein 1994).

Amoxicillin showed shorter growth suppression and PAE in comparison with fluoroquinolones, especially with *P. multocida*. An increase in concentration and exposure time had no significant effect on final PAE (see Tables 5 and 7). No increase of the PAE was seen when the concentration of amoxicillin was increased further in agreement with published data (Thorburn et al. 1996). Short PAE durations were noted for *P. multocida* isolates up to 2 h, in agreement with other reports on Gram-positive bacteria (Gerber and Craig 1981; Craig and Vogelman 1986; Thorburn et al. 1996).

Different levels of PAE in β-lactams in comparison with fluoroquinolones were expected, as it is generally observed that β-lactams do not elicit PAE on Gram-negative bacteria. However, we observed moderate effects on isolates of *H. parasuis* and rather long PAEs on isolates of *A. pleuropneumoniae*, which was comparable with that elicited by fluoroquinolones. Similarly, a long PAE was described as well for *H. influenzae* isolates and amoxicillin-clavulanate (Thorburn and Moleswoth 1992). This observation might explain the already described strong sub-MIC effect of amoxicillin in an *in vitro* and *in vivo* study where it prevented 100% mortality of pigs in controls challenged by *A. pleuropneumoniae* and non-treated groups (Tanigawa and Sawada 2002).

Antibiotics that exert PAE allow the use of lower antibiotic concentrations below the MIC for significant periods of time without risk of re-growth and consequently the administration of antibiotics in an intermittent dose (Carbone et al. 2001). Post-antibiotic sub-inhibitory concentration effect may play a role as well, and this effect was described for fluoroquinolones (Harada et al. 2012). Differences in PAEs were also evident between bacterial strains of the same origin. The lowest variability was de-

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tected for marbofloxacin. *A. pleuropneumoniae* isolates revealed the best PAE irrespective of the antimicrobial tested (the range of means of PAE for amoxicillin 4–8.5 h; enrofloxacin 8.3–10.2 h; marbofloxacin 8.3–10.3 h), followed by *H. parasuis* (amoxicillin 3.6–4.2 h; enrofloxacin 4.6–8 h; marbofloxacin 6.5–8.8 h). In all three antibiotics, the markedly shortest duration was recorded for *P. multocida* (amoxicillin 1.3–1.7 h; enrofloxacin 3.5–6.5 h; marbofloxacin 5.1–6.3 h).

Hossain et al. (2017) evaluated selected pharmacokinetic and pharmacodynamic profiles of marbofloxacin in pig against *A. pleuropneumoniae* isolates. The elimination half-life ($T_{1/2}$) of marbofloxacin in pigs for *A. pleuropneumonia* isolates was 8.6 ± 0.3 h, 12.8 ± 1.10 h, and 8.6 ± 0.0 h, respectively, in response to intravenous (*i.v.*), intramuscular (*i.m.*) and peroral (*p.o.*) administration. Similarly, an *ex vivo* antibacterial effect in plasma samples was observed within the first 12 h of incubation after *i.v.* and *i.m.* administration of drug. We demonstrated comparable *in vitro* duration of PAE for marbofloxacin, from eight to 12 hours for *A. pleuropneumoniae* isolates (see Table 3).

There is conflicting evidence about the effect of MIC on PAE. Some studies show a negative effect of increased MICs of tested antibiotics (enrofloxacin), showing improvement once sensitive strains are used (Harada et al. 2012). In contrast, in our study and some others (Licata et al. 1997), several isolates of the same pathogen with the same MIC values showed different PAEs. Thus, the duration of growth suppression might be dependent on additional factors along with the MIC. These results were consistent with a previously published report (Zhao et al. 2014). Variability might also depend on factors like the growth rate of the culture, as a certain level of variability might be present during testing. For a better understanding, additional complementary evaluation might also be considered, e.g., evaluation of the morphology of bacteria.

Marbofloxacin showed the best results in our study. Enrofloxacin showed PAE of comparable duration in *A. pleuropneumoniae* and *H. parasuis*, but for *P. multocida* isolates, PAE was significantly shorter. Amoxicillin showed shorter PAE in all three pathogens compared with fluoroquinolones as expected. On the other hand, the duration of PAE, especially for *A. pleuropneumoniae* was longer than commonly reported for other Gram-negative bacteria and might have clinical importance. For the first time, we have confirmed a prolonged PAE of fluoroquinolones and amoxicillin in important Gram-negative swine pathogens. The increasing levels of resistance in bacteria and the lack of new veterinary antimicrobial agents on the horizon means that more research is needed to clarify the effect and mechanism of action of antimicrobial drugs.

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